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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

Stromal-epithelial interactions regulate mammary epithelial cell (MEC) fate via integrin-growth factor receptor (GFR) interactions. Integrin-GFR crosstalk influences MEC behavior through activation of tyrosine kinase signaling that is tempered by protein tyrosine phosphatase (PTP) activity of which we know little about. Using a degenerate RT-PCR to amplify PTPs expressed in differentiated versus non-differentiated MECs, we identified the Band 4.1 PTPs MEG1 and D1 as two candidate PTP metastasis suppressors. Our studies show that during MEC differentiation PTP MEG1 and D1 expression rise dramatically, coincident with assembly of E-cadherin/β-catenin adherens junction formation. However, both mRNA and protein expression of MEG1 and D1 become repressed following MEC tissue differentiation. Because we could not establish any correlation between MEC growth, or tissue polarization, this suggests that MEG1 and D1 expression may be functionally linked to adherens junction assembly. Consistently, malignant MECs that fail to assemble adherens junctions do not modulate MEG1 or D1 expression. Moreover, MEG1 expression is not induced in phenotypically reverting tumors, or down regulated in dormant structures. This suggests that these Band 4.1 PTPs may be functionally-linked to molecules mediating adherens junction formation.

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INTRODUCTION:

Stromal-epithelial interactions and integrins regulate mammary gland development and homeostasis, and modulate tumor progression and treatment responsiveness [1, 2]. Although it is not known how the stroma exerts such a profound effect on mammary epithelial cell (MEC) function, studies in culture have demonstrated that the basement membrane (BM) extracellular matrix (ECM); (the insoluble protein component of the stroma surrounding MECs in vivo) regulates MEC growth, survival, migration and differentiation through transmembrane receptors called integrins [3]. Integrins mediate their biological effects by initiating biochemical and biophysical signaling that involves protein tyrosine phosphorylation events, and that also rely upon cooperative interactions with growth factor receptors and cytoskeletal interactor/modifier molecules [4, 5]. The level of tyrosine phosphorylation induced by integrin and growth factor receptor activation is dynamically regulated by the concerted action of protein tyrosine kinases and protein tyrosine phosphatases (PTPs). Several lines of evidence suggest that both transmembrane and intracellular PTPs play an important role in mammary morphogenesis [6, 7]. Indeed, culture studies have implicated PTPs as critical regulators of cell-cell interactions [8], integrin-mediated signaling [9, 10], and focal adhesion assembly [11], and as modifiers of integrin-growth factor receptor interactions [4, 9]. However, very little is known about the molecular functions and substrate targets of PTPs in adhesion-dependent MEC behavior. Thus, the overall goal of our studies is to delineate the importance of and identify plausible mechanisms whereby PTPs regulate, and are themselves regulated by ECM-integrin-growth factor receptor signaling, and thereafter to determine the role of PTPs in breast cancer progression.

To achieve our goal we have been studying normal and perturbed morphogenesis of MECs using a human breast cancer model called HMT-3522, which progresses from non-malignant S-1, through pre-malignant S-2, to tumorigenic T4-2 MECs [12], as well as using a non-malignant immortalized MEC line established from a fibrocystic breast lesion called MCF10A [13]. When used in conjunction with a reconstituted BM assay these MECs recapitulate the morphological and behavioral features of MECs as they transit from a non-transformed, through to a pre-malignant, and thereafter to a malignant, invasion breast tissue phenotype. Using these MEC lines our studies have clearly established that the progressive malignant behavior of the cells in this series is linked to alterations in integrin-growth factor expression and activity, and perturbed MEC-ECM responsiveness, because we are able to phenotypically revert our mammary tumor cells in culture, and in vivo, by inhibiting the activity of either β1 integrin and/or the epidermal growth factor receptor [1, 14]. Alternately, we can drive the malignant behavior of non-malignant and the pre-malignant MECs by altering the expression of specific integrin heterodimers such as α5β1 and α6β4 integrin ([15] [16] and Weaver, unpublished observations).

We successfully identified 15 PTPs whose mRNA levels appear to be regulated during adhesion-directed morphogenesis in these MECs, and that become altered following malignant transformation. In particular, we noted that members of the Band 4.1 PTPs including PTP Megl, D1, H1 and FAP are dramatically down regulated following MEC differentiation, and that their expression is increased in the tumors. Preliminary studies indicated that PTP Meg 1 and D1 are significantly increased 24 hours following the interaction of nonmalignant MECs with a reconstituted BM, and prior to differentiation, whereas the tumors do not appear to regulate these PTPs in response to ECM cues. These two PTPs have been strongly implicated as key regulators of cell adhesion, formation of focal adhesion and cytoskeletal organization, and their dysregulation has been implicated in uncontrolled proliferation and altered cell-ECM interactions [17-20], and they appear to be robustly regulated during normal mammary morphogenesis in the developing mammary gland in vivo. Accordingly, we have formulated the working hypothesis that: **PTP Megl and D1 play pivotal roles in ECM directed MEC morphogenesis, and that their dysregulation contributes to malignant transformation and acquisition of an invasive, metastatic breast tumor phenotype.**

We are now attempting to test this hypothesis by achieving the following specific aims:

Specific Aim 1: Generate PTP-Meg 1 and D1 antibodies and then characterize the expression, activity and localization of these endogenous PTPs in normal and dyregulated BM-directed MEC morphogenesis, and identify the morphological parameters linked to their regulation.

Specific Aim 2: Analyze the cellular and molecular consequences of over expression of PTP-Meg 1 and D1 wild type and dominant negative mutants during tumor progression and following phenotypic reversion.

Specific Aim 3: Identify and characterize known and/or novel PTP-Meg 1 and D1 substrates/binding proteins and/or cellular targets that may lie on pathways implicated in Specific Aims 1 and 2.

Summary of Achievements - Proposal Body:

1. Characterization of PTP Dynamics during MEC Morphogenesis:

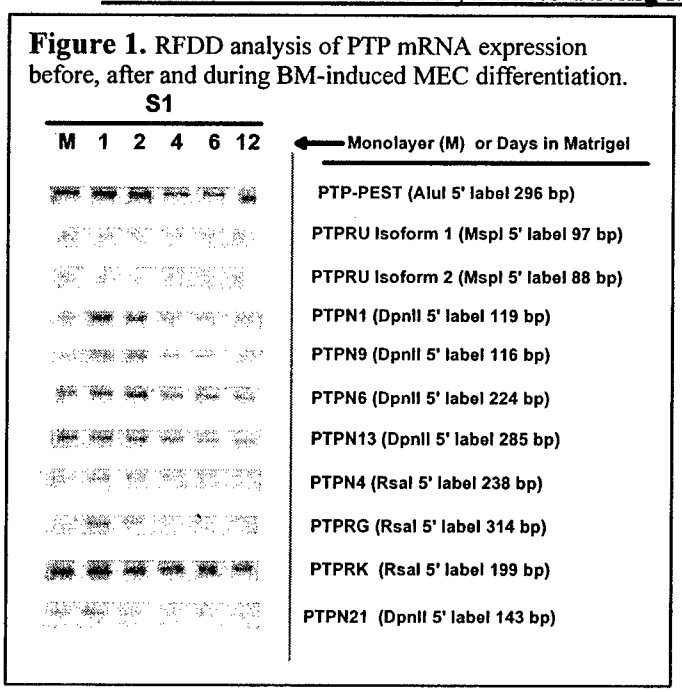
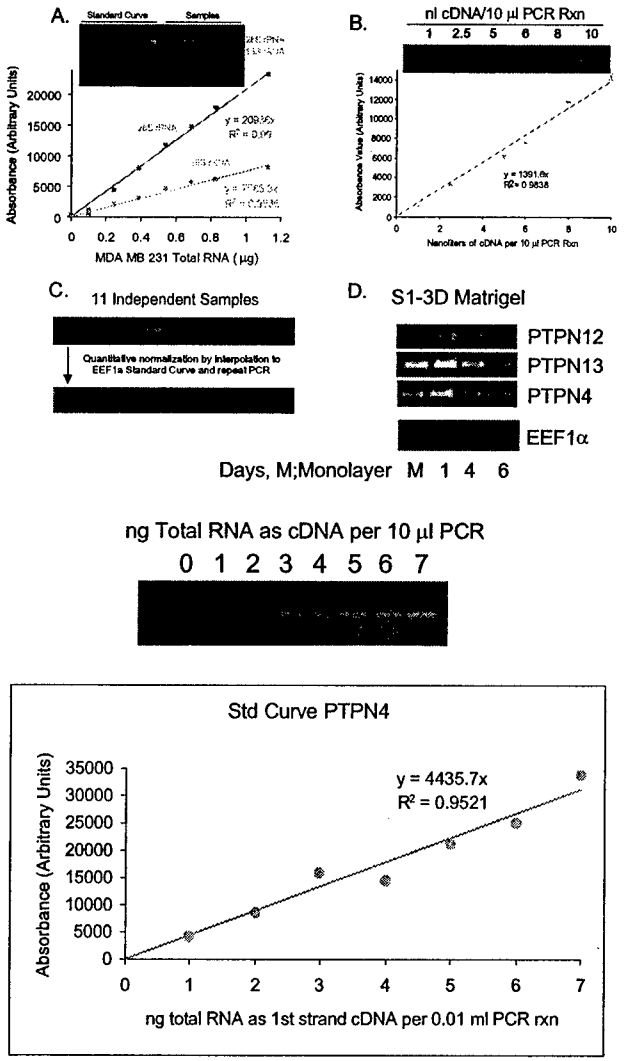


Figure 1. RFDD analysis of PTP mRNA expression before, after and during BM-induced MEC differentiation.

Our first goal was to verify our preliminary data that indicated that PTP Meg 1 and D1 mRNA expression is down regulated following MEC morphogenesis, and thereafter to determine what morphological parameters linked to epithelial morphogenesis might be associated with their regulation. To achieve this goal we prepared total RNA from multiple experiments of nonmalignant MECs before and after BM-induced differentiation and profiled the expression of PTPs using our restriction fragment differential display method. RFDD of reverse transcriptase-polymerase chain reaction (RT-PCR) amplification of total standard PTP catalytic domains of total RNA prepared from non-malignant HMT-3522 S1 and MCF10A non differentiated (growth-arrested, MECs grown for 10-16 days as standard monolayers on tissue culture plastic) and differentiated MECs (growth-arrested MECs grown for 10-16 days within a reconstituted basement membrane) was achieved using degenerate primers to conserved sequences of PTP domains. In this analysis individual PTPs are identified on the basis of different 4 base pair restriction sites in non-conserved sequences of the RT-PCR products that are then separated on a denaturing gel and visualized by autoradiography. We have used this approach successfully to identify these two Band 4.1 PTPs as candidate PTPs for additional follow up studies, and methods are already in place to identify that bands correspond to Meg 1 (PTP N4) and D1 (PTP N21). We conducted three independent sets of mammary differentiation studies and thereafter analyzed changes in PTP expression using RFDD followed by scanning densitometry for quantitative analysis. Results clearly showed that both Band 4.1 PTP Meg 1 or N4 and D1 or N21 are significantly lower in MEC following their assembly into differentiated breast acini (See Figure 1).

1. Generation of biochemical tools for PTP Meg 1 and D1 analysis:

Figure 2. RT-PCR Standardization assay with validation of EEF1a as an internal control and establishment of semi-quantitative of PTP Meg1.

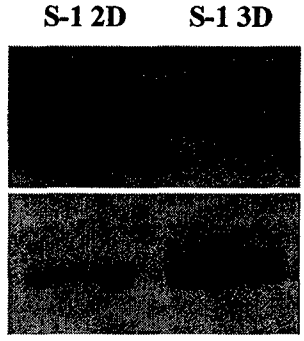


Having verified our initial preliminary results our next goal was to develop the appropriate tools with which to rigorously assay for changes in Band 4.1 PTP Meg 1 and D1 RNA, protein and activity levels during normal and dysregulated mammary tissue morphogenesis. To achieve this goal we established a semi-quantitative RT-PCR assay for PTP expression in small tissue samples that included a systematic validation of an array of internal controls (see Figure 2). After much analysis we were able to identify EEF1a as a valid internal control that does not change during epithelial differentiation, nor in response to differences in ECM microenvironment, and that does not become altered in tumors or change as a function of cell proliferation or apoptosis. The utility of EEF1a as a reliable internal target has since been highlighted by other investigators and is now routinely used as a standard control for micro array expression experiments, thereby demonstrating the prudence of our decision to standardize this molecule for our assays. To date we have successfully applied the RT-PCR method, normalizing our sample RNA levels to EEF1a, to validate our RFDD results. RT-PCR results comparing multiple differentiated versus non-differentiated nonmalignant MECs, clearly showed that PTP Meg 1 mRNA expression is strongly down regulated in MECs following tissue differentiation (see Figure 2). Studies are currently under way to achieve similar results with PTP D1.

Our institution has recently purchased a LightCycler (Roche) that will enable QPCR to be performed by broadly accepted approaches. The application of QPCR should afford easier assessment of total mRNA levels of PTP Meg 1 and D1. Because QPCR is highly reproducible and quantitative and can be easily conducted on small amounts of isolated RNA, we have expended considerable effort to adopt the LightCycler methodology for our 3D BM assay studies. In this regard, we have successfully set up the system and validated the EEF1a as a viable internal control, and have successfully applied this method to precisely quantify the levels of other mRNAs including SMRT. We anticipate that this next year we will set up and begin to routinely use this QPCR procedure for our PTP work.

An objective of our work is to generate monoclonal antibodies against PTP Meg 1 and D1 that can be used for biochemical and immunofluorescence studies. To this end we have acquired full length pPTPMEG, mutant (activity dead) pPTPMEGCS, and truncated pPTPMEG1-647H, pPTPMEG1-367H, and pPTPMEG368-647H baculovirus expression constructs and we are in the process of generating recombinant PTPMEG protein for antibody generation and biochemical studies. Our goal is to generate

Figure 3. Immunoblot analysis of Meg 1 protein in MECs before and after tissue differentiation.

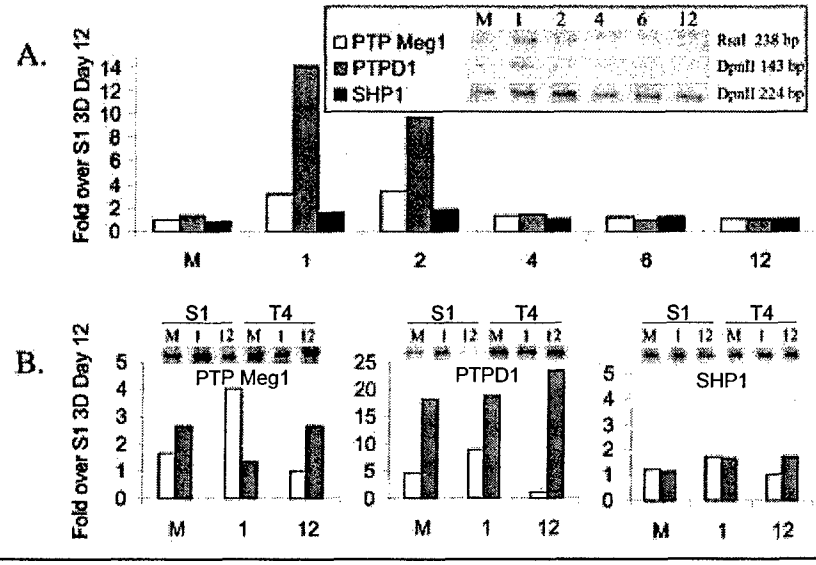


antibodies using Phage Display. Towards this goal we have procured large diversity naive human synthetic scFv phage display Tomlinson I and J libraries which we have recently expanded and will begin screen against recombinant PTPMEG proteins. As an alternate strategy however, and in response to the grant reviewers advice and comments, once we have obtained sufficient quantities of semi-purified recombinant protein we believe that it will be prudent to also attempt to generate polyclonal antibodies through an outside facility. In the meantime, we were lucky enough to be able to secure small amounts of polyclonal antibodies to the full length, C-terminus and the N-terminus of the PTPMEG1 protein. Using these antibodies we have successfully demonstrated that PTP Meg 1 protein levels accord reasonably well with mRNA expression levels, and that PTP Meg 1 protein expression is also down regulated following MEC differentiation (see Figure 3). Studies are now underway to use this antibody to assess whether mammary tissue differentiation is also associated with down regulation of PTP Meg 1 activity.

3. Characterization of PTPMeg1 during MEC Morphogenesis:

Preliminary experiments suggested that although PTP Meg 1 and D1 are significantly down regulated in differentiated MEC tissues, they are robustly and highly up regulated in MECs 24 hours following epithelial cell embedment within a reconstituted basement membrane. This suggests that PTP Meg 1 and D1 may play a critical role in directing MEC morphogenesis, but that once a differentiated structure is formed they are down regulated and no longer required for tissue homeostasis. Repeat RFDD experiments validated these preliminary experiments and clearly showed that Meg 1 and D1 mRNA is up regulated rapidly and prior to acquisition of a differentiated stable tissue (see Figures 1, 4 & 8).

Figure 4. Quantification of PTP expression changes during MEC tissue differentiation.



In an effort to define what role Meg 1 and D1 might play in MEC morphogenesis we correlated acquisitions of cell-cell interactions (assay for cell-cell localized beta catenin; and apical lateral ZO-1 localization.) and tissue polarization (basal deposition of laminin 5 and collagen IV) to changes in Meg 1 and D1 expression. We also assayed for at relationship between Meg 1 and D1 expression and changes in MEC proliferation by assay BrDU incorporation (data not shown) and Ki67 expression (see Figure 7). Our experimental data clearly showed that Meg 1 and D1 mRNA are increased prior to development of stable cell-cell interactions and acquisition of tissue polarization (see Figure 5 & 6), and that levels of Meg 1 and D1 decrease long before MECs cease to proliferate (see Figure 7). These data suggest that Meg 1 and D1 may be important for and in turn regulated by establishment of stable cell-cell interactions and/or tissue

Figure 5. Time course of adherens junction assembling during MEC tissue morphogenesis as indicated by ZO-1 and beta catenin localization at cell-cell junctions.

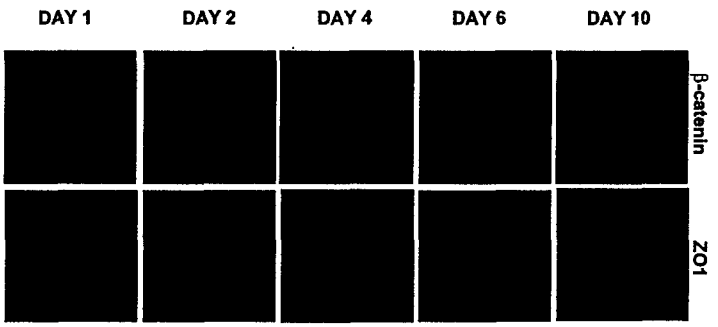


Figure 6. Time course of tissue polarity and basement membrane assembly during MEC tissue morphogenesis as indicated by collagen IV and laminin 5 deposition around the basal domain of the tissue.

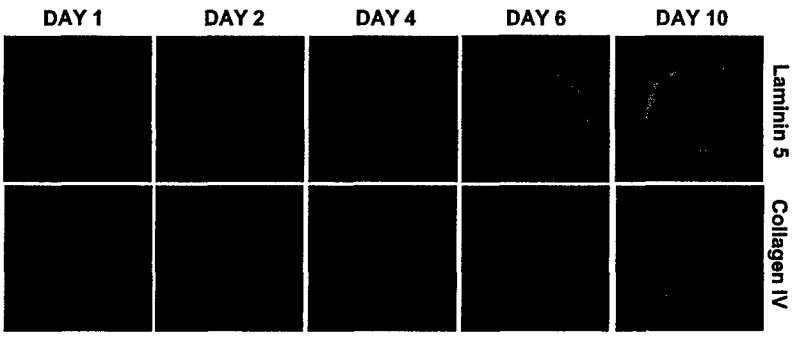
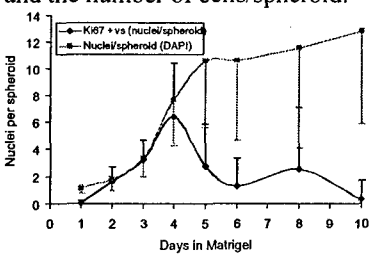


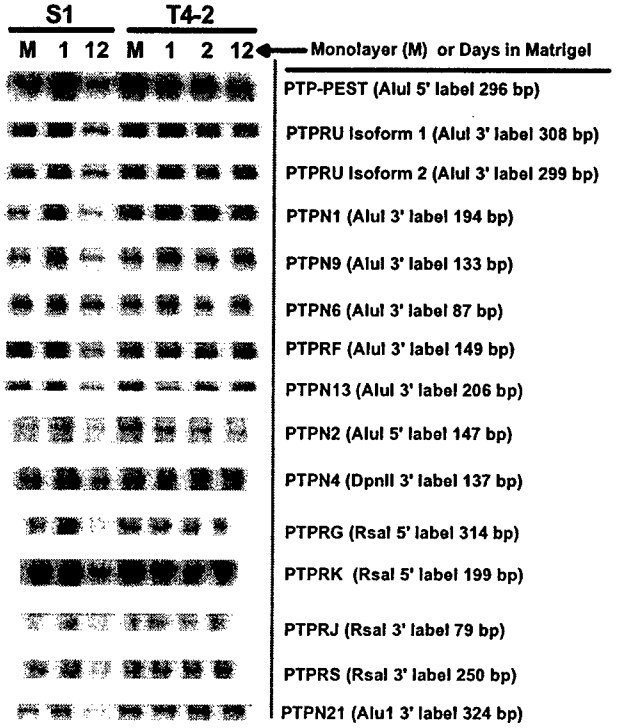
Figure 7. Time course of MEC growth response during BM-induced tissue differentiation as quantified by Ki67 expression and the number of cells/spheroid.



polarization, but that they appear not to be functionally-linked to cell proliferation. To verify whether or not PTP Meg 1 and D1 regulate or are themselves regulated by cell proliferation we are studying regulation of Meg 1 and D1 in nonmalignant MECs stably expressing an activated form of the epidermal growth factor receptor (EGFR) or erbB2 [13, 21]. Studies are now underway to determine whether or not changes in mRNA expression are also paralleled by alterations in protein expression and PTP activity.

To explore links to tissue polarization we embedded nonmalignant MECs within collagen I gels, that do not support tissue polarization in the absence of $\alpha 6\beta 4$ integrin ligation [22]. Because RFDD performed on RNA isolated from 12-day cultures of non-polarized MEC spheroids grown within collagen gels showed that Meg 1 and D1 PTPs were again down regulated, it seems highly unlikely that the expression of these PTPs is linked to pathways regulating tissue polarization. However, to verify this conclusion, additional experiments are being performed on nonmalignant MECs that express a tailless $\beta 4$ integrin that will perturb tissue polarization in MECs grown within a reconstituted BM [22]. Instead, these data indicate that PTP expression may be functionally linked to pathways involved in directing the formation of stable cell-cell adherens junctions. To explore links to formation of stable cell-cell interactions we have established stable populations of S-1 and MCF10A nonmalignant MECs that express dominant negative versions of E cadherin and experiments are under way to explore effects on Meg 1 and D1 expression in these MECs as a function of BM-directed morphogenesis (Murine MHC Class I H-2Kd extracellular domain fused to transmembrane and cytoplasmic domains of human E cadherin; [23].

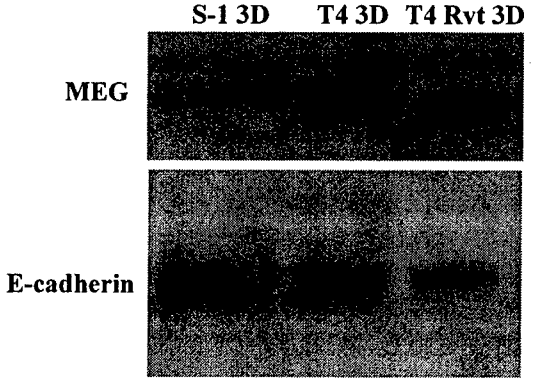
Figure 8. RFDD analysis of PTP mRNA profiles expressed in nonmalignant and transformed MECs embedded within rBM to induce tissue differentiation.



Characterization of PTP Meg 1 during mammary tumor progression:

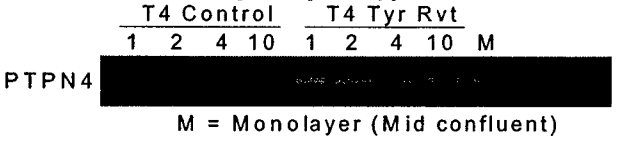
Our initial studies showed that Meg 1 and D1 mRNA levels were elevated in mammary tumors as compared to differentiated MEC acini. Data also suggested that while Meg 1 and D1 levels rose appreciably within the first 24 hours following embedment within either a reconstituted BM or inside of floating collagen I gels, levels remained constant in the tumors. We have now verified these data using RFDD, RT-PCR and western immunoblot analysis. RFDD and RT-PCR clearly showed that levels of Meg 1 and D1 do not change in tumors following their interaction with a reconstituted BM, and are not down regulated even after 12-16 days of rBM growth (see Figures 8, 9 & 10). More importantly, although the levels of PTP Meg1 and D1 are higher in the tumor colonies as compared to the differentiated MEC acini, Meg 1 and D1 levels never transiently rise to achieve levels expressed in the nonmalignant MECs in the first 24-96 hours, prior to adherens junction formation (see Figures 5 & 8). Because the T4-2 MECs from the HMT-3522 series do NOT form stable cell-cell adherens junctions these data are consistent with our current hypothesis that Meg 1 and/or D1 PTPs may be functionally linked to pathways involved in driving the formation of stable adherens junctions.

Figure 9. Immunblot analysis of Meg 1 protein changes in differentiated nonmalignant MECs and their tumorigenic progeny before and after phenotypic reversion.



To address whether PTP Meg 1 or D1 regulate adherens junctions or are critical for adherens junction formation, we assayed for changes in PTP Meg 1 and D1 expression in malignant MECs during T4-2 phenotypic reversion. T4-2 MECs embedded within BM gels were treated with either a function-blocking antibody to β1 integrin or tyrophostin, which is a specific inhibitor of EGFR signaling, and total RNA or protein was isolated and subjected to RFDD and RT-PCR mRNA analysis and western immunoblot assays. Interestingly, results showed that phenotypic reversion of T4-2 MECs failed to alter mRNA expression of PTP Meg 1 and D1. Thus, neither PTP Meg 1 nor D1 mRNA levels changed appreciably in response to ligation of ECM receptors or following MEC morphogenesis in the case of the phenotypically reverted tumors (see Figure 10). Although, immunoblot results did indicate that phenotypic reversion did repress PTP Meg 1 expression appreciably (see Figure 9). These data suggest that Meg 1 or D1 likely functionally upstream

Figure 10. RT-PCR analysis of PTP Meg 1 in tumor tissues before, after and during their phenotypic reversion.



of pathways that are critical for formation of a differentiated tissue structure, including those regulating the assembly of stable adherens junctions, although we have yet to rule out the possiblity that formation of a stable tissue structure might itself alter PTP Meg 1 expression. The relevance of these observations will need to be clarified in the next two years and should be greatly facilitated following assessment of changes in PTP activity and subcellular localization in differentiated, tumor and phenotypically reverted MEC tissues, as well as following the identification of PTP Meg 1 and D1 cellular substrate targets.

Generation of molecular tools for manipulating PTP Meg 1 and D1:

To delineate the relevance of changes in PTP Meg 1 and D1 and normal and malignant mammary epithelial tissue organization we have obtained PTP Meg 1 wild type and phosphatase dead cDNA expression constructs [24, 25]. Because BM-induced MEC morphogenesis is a well timed and coordinated sequence of events and PTP Meg 1 and D1 may function at specific points in this process (see above), we are in the process of sub cloning these cDNA expression constructs into the RetroTet ART tripartite tetracycline (Tet) inducible retroviral expression system [26]. Moreover, because our morphogenesis assays are clonal in nature and exogenously expressed retroviral expressed proteins often show variable expression levels, we have decided to create EGFP-tagged expression constructs so that we can identify those colonies that express high levels of the expressed proteins and correlate morphological changes with expression levels. These techniques are well established in my laboratory and therefore we do not anticipate encountering any unforeseen difficulties with these procedures. An additional advantage of using tagged proteins is that they permit easy visualization of the subcellular localization of expressed protein, permitting facile verification of results obtained by immunolocalization experiments.

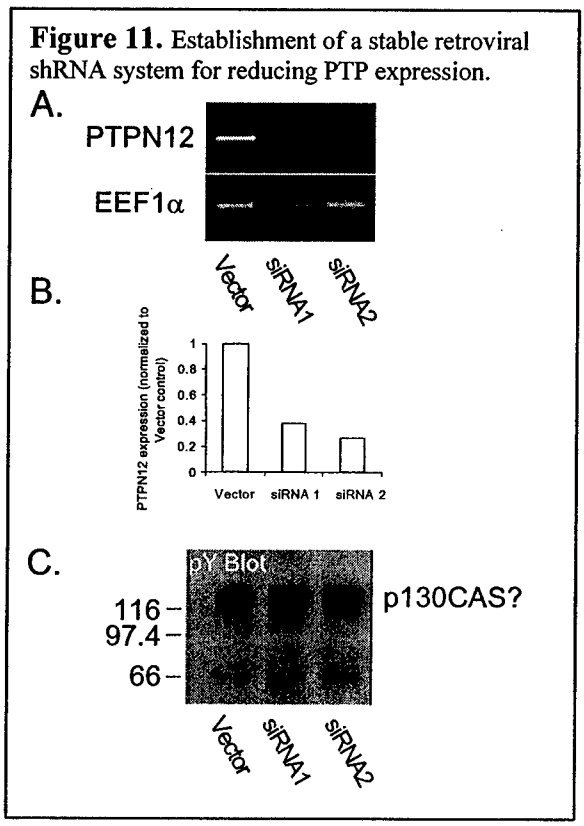


Figure 11. Establishment of a stable retroviral shRNA system for reducing PTP expression.

To augment these studies we have also obtained candidate siRNA retroviral expression constructs for PTP Meg 1 which will be used to study the effect of knocking down Meg 1 levels on MEC morphogenesis, tumorigenesis and phenotypic reversion. This technique has proved especially useful for assessing the functional importance of another adhesion regulated PTP - PTP-PEST in MEC morphogenesis and tumorigenesis (see Figure 11). As an additional strategy we have set up an adenoviral expression system in the laboratory that will afford us the ability to over-express wild type and dominant-negative PTP Meg 1 and D1 in the differentiated acini and in the malignant colonies. Finally, towards identifying candidate PTP Meg 1 and D1 substrates we are currently designing inducible GST-tagged PTP Meg 1 expression constructs. In summary then, we are confident that we will have these tools in hand within the next 4-6 months and should be able to generate stable nonmalignant, pre-malignant and malignant MEC lines for functional studies.

KEY RESEARCH ACCOMPLISHMENTS:

- RFDD confirmation studies of PTPMeg1 and D1 differences between differentiated and non-differentiated nonmalignant MECs.
- Semi-quantitative RT-PCR method for PTP Meg1 set up and validated
- Semi-quantitative RT-PCR validation of RFDD PTP Meg1 observations
- Procurement of polyclonal PTPMeg1 antibody
- Verification of RFDD and RT-PCR PTP Meg1 results using PTP Meg1 antibody and western immunoblot analysis
- Characterization of PTP Meg1 expression using RFDD and RT-PCR during MEC morphogenesis
- Association between PTP Meg1 expression and MEC proliferation explored
- Association between PTP Meg1 expression and adherens junction formation in differentiated MEC acini explored and demonstrated
- Association between PTP Meg1 expression and polarization of MEC acini explored
- RFDD Analysis of PTP Meg1 & D1 in tumors and in phenotypically reverting tumors
- RT-PCR verification of PTP Meg1 expression in normal, tumor and phenotypically reverted MEC tumor colonies
- Western immunoblot analysis of PTP Meg1 expression in normal, tumor and phenotypically reverted MEC tumor colonies
- Procurement of PTP Meg1 wild type and mutant expression constructs and generation of RetroTet ART PTPMeg1 wild type and dominant-negative expression constructs
- Establishment of stable siRNA retroviral protocols and the procurement of candidate PTP Meg1 siRNAs for PTP Meg1
- Establishment and expansion of Phage Display Libraries for future generation of PTP Meg1 and D1 monoclonal antibodies
- Procurement of PTP Meg1 constructs for generating recombinant expressed PTP Meg1 protein for generation of PTP Meg1 antibodies and for pull down experiments aimed at identifying candidate substrates
- Establishment of adenoviral expression system for manipulation of PTP expression in differentiated MEC acini

Reportable Outcomes:

A. Manuscripts

1. Lakins, J.N., Chrenek, M., Kang, B., Wong, P., and Weaver, V.M. Adhesion-linked protein tyrosine phosphatases and tissue morphogenesis. In Revision.

2. Paszek, M.J., Zahir, N., Lakins, J.N., Lynch, L., Johnson, K., Rozenberg, G.I., Reinhart-King, C., Beg, J., Dembo, M., Boettiger, D., Hammer, D.A. and Weaver, V.M. Spatial-mechanical regulation of epithelial morphogenesis and transformation. Submitted.

B. Abstracts

1. Johnathon N. Lakins and Valerie M. Weaver, RNA interference of PTPN12 enhances acinar growth of MCF10A cells potentially through activation of Rho. ASCB, Washington, D.C. December, 2004.

C. Oral Meetings Presentations:

1. Weaver, V.M., The tension mounts: mechanics meets morphogenesis, ASCB, Special Session on Integrated approaches to cell architecture and dynamics, San Francisco, CA., December 13, 2003

2. Weaver, V.M., ECM-dependent reversibility of the malignant phenotype, AACR Symposium on the Reversibility of Tumorigenesis, Orlando, Florida, March 28, 2004.

3. Weaver, V.M., Spatial and biochemical regulation of malignant transformation of mammary gland, Radiation Biology Conference, St. Louis, MS, April 27, 2004.

4. Weaver, V.M., Outside in Signaling: The importance of the Breast Tissue Microenvironment, Third Annual Future of Breast Cancer: An International Congress, New concepts in Eradicating Breast Cancer - Novel and Established Growth, Survival Mechanisms, and Clinical Implications, Hamilton, Bermuda, July 24, 2004.

D. Invited Institutional Presentations:

1. Weaver, V.M., Spatial-mechanical modulation of morphogenesis and malignancy, Vanderbilt-Ingram Cancer Center, Nashville, TN, January 6, 2004.

2. Weaver, V.M., The Epigenetics of breast cancer progression: deciphering the role of stromal-epithelial interactions, Center for Molecular Studies in Digestive and Liver Disease Research, University of Pennsylvania, Philadelphia, PA, January 22, 2004.

3. Weaver, V.M., Spatial-Mechanical Regulation of ECM-directed Morphogenesis and Malignancy, Baylor College of Medicine, Houston, TX, February 4, 2004.

4. Weaver, V.M., Tension in the Neighborhood: Mechanics meets Morphogenesis, McArdle Laboratory for Cancer Research, University of Wisconsin-Madison, Madison, Wisconsin, February 18, 2004.

5. Weaver, V.M., Mechanics meets morphogenesis and malignancy", McGill University, Montreal, Que, CANADA, March 11, 2004.

6. Weaver, V.M., The tension mounts: Mechanics meets morphogenesis and malignancy, University of Virginia, Charlottesville, VA, March 26, 2004.

7. Weaver, V.M., Tension in the neighborhood: microenvironmental mechanics meets mammary morphogenesis and malignancy, Department of Surgery Grand Rounds, UCSF General Hospital, San Francisco, CA, April 13, 2004.

8. Weaver, V.M., Tension in the neighborhood: microenvironmental mechanics meets mammary morphogenesis and malignancy, UCSF Cancer Center Breast Cancer SPORE meeting, San Francisco, CA, April 14, 2004.

9. Weaver, V.M., Peer Pressure: microenvironmental mechanics meets mammary morphogenesis and malignancy, Biomedical Sciences Symposium, UCSF Parnassus Campus, San Francisco, CA, April 14, 2004.

10. Weaver, V.M., Spatial-mechanical regulation of apoptosis during morphogenesis and tumor progression, University of Toronto, Toronto, Ontario, CANADA, December 8, 2003.

Conclusions:

In this first performance year, funding from the DOD Era of Hope BCRP foundation has enabled my laboratory to develop several tractable tools that we are now actively using to study the role of the PTPs Meg1 and D1 in mammary morphogenesis and tumor progression. These tools include the set up and validation of improved semi-quantitative RT-PCR and a quantitative PCR based light cycler method for assessment of PTP mRNA expression in small samples that incorporates a validated reproducible internal control for reproducible analysis of PTP expression, and the establishment of stable retroviral siRNA and adenoviral expression systems. We have also initiated work to generate inducible EGFP-tagged retroviral wild type and dominant-negative PTP Meg1 expression constructs, procured and validated polyclonal antibodies for Meg1, and began studies to develop phage display monoclonal antibodies to PTP Meg1 and D1 for immunohistochemistry work. Using our previously established methods and these newly developed tools and protocols we have been able to verify our preliminary RFDD observations so that we can definitively conclude that MEC tissue differentiation leads to down regulation of Band 4.1 PTP Meg 1 and D1 mRNA expression, and have additionally confirmed these results using semi-quantitative RT-PCR and RNase protection assays. We obtained a polyclonal antibody against the human band 4.1 PTP Meg1 and showed that changes in MEC mRNA expression are reflected by similar changes in PTP protein expression. Studies are now underway to generate better polyclonal and monoclonal PTP Meg 1 antibodies that can be used for co-IPs and immunohistochemistry studies to assess PTP substrates and monitor subcellular localization in our tissue culture models and in tissue samples from animals and humans. By conducting a careful morphological analysis of non-malignant, transformed and phenotypically reverted tumors, we were able to demonstrate that there is likely a functional link between acquisition of stable cell-cell adherens junctions and the band 4.1 PTPs Meg 1 and D1, and that these Band 4.1 PTPs appear to function up stream of pathways facilitating adherens junction formation. Additional experiments are now underway using a variety of activated EGFR and ErbB2, and mutant E cadherin expressing nonmalignant MECs to

more rigorously investigate this possibility. Thus in summary, we conclude that the Band 4.1 PTPs Meg 1 and D1 may constitute attractive candidate "tumor suppressor or metastasis suppressor" molecules that merit further study. It is our hope that in the next year we will be able to carefully manipulate PTP Meg 1 and/or D1 expression and/or activity and identify some of their candidate subcellular targets so that we will be able to clarify the role(s) of these PTPs in ECM directed MEC tissue function and breast tumorigenesis.

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